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I 171 173  
B  
Cont  
acid. For example, huTNF-RA235 refers to human TNF-R having Asp<sup>235</sup> as the C-terminal amino acid (i.e., a polypeptide having the sequence of amino acids 1-235 of [Figure<sup>FIG.</sup> 2A] SEQ ID NO:1). In the absence of any human or murine species designation, TNF-R refers generically to mammalian TNF-R. Similarly, in the absence of any specific designation for deletion mutants, the term TNF-R means all forms of TNF-R, including mutants and analogs which possess TNF-R biological activity. --

Page 6, lines 1-21, delete in their entirety, and insert therefor

I 171 173  
Dx  
-- "Soluble TNF-R" or "sTNF-R" as used in the context of the present invention refer to proteins, or substantially equivalent analogs, having an amino acid sequence corresponding to all or part of the extracellular region of a native TNF-R, for example, huTNF-RA235, huTNF-RA185 and huTNF-RA163, or amino acid sequences substantially similar to the sequences of amino acids 1-163, amino acids 1-185, or amino acids 1-235 of [Figure<sup>FIG.</sup> 2A] SEQ ID NO:1, and which are biologically active in that they bind to TNF ligand. Equivalent soluble TNF-Rs include polypeptides which vary from these sequences by one or more substitutions, deletions, or additions, and which retain the ability to bind TNF or inhibit TNF signal transduction activity via cell surface bound TNF receptor proteins, for example huTNF-RAx, wherein x is selected from the group consisting of any one of amino acids 163-235 of [Figure<sup>FIG.</sup> 2A] SEQ ID NO:1. Analogous deletions may be made to muTNF-R. Inhibition of TNF signal transduction activity can be determined by transfecting cells with recombinant TNF-R DNAs to obtain recombinant receptor expression. The cells are then contacted

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B7  
Cont

with TNF and the resulting metabolic effects examined. If an effect results which is attributable to the action of the ligand, then the recombinant receptor has signal transduction activity. Exemplary procedures for determining whether a polypeptide has signal transduction activity are disclosed by Idzerda et al., *J. Exp. Med.* 171:861 (1990); Curtis et al., *Proc. Natl. Acad. Sci. USA* 86:3045 (1989); Prywes et al., *EMBO J.* 5:2179 (1986) and Chou et al., *J. Biol. Chem.* 262:1842 (1987). Alternatively, primary cells or cell lines which express an endogenous TNF receptor and have a detectable biological response to TNF could also be utilized. --

[ Page 8, lines 16-28 delete in their entirety, and insert therefor

B23

-- The coding sequence of TNF-R is obtained by isolating a complementary DNA (cDNA) sequence encoding TNF-R from a recombinant cDNA or genomic DNA library. A cDNA library is preferably constructed by obtaining polyadenylated mRNA from a particular cell line which expresses a mammalian TNF-R, for example, the human fibroblast cell line WI-26 VA4 (ATCC CCL 95.1) and using the mRNA as a template for synthesizing double stranded cDNA. The double stranded cDNA is then packaged into a recombinant vector, which is introduced into an appropriate *E. coli* strain and propagated. Murine or other mammalian cell lines which express TNF-R may also be used. TNF-R sequences contained in the cDNA library can be readily identified by screening the library with an appropriate nucleic acid probe which is capable of hybridizing with TNF-R cDNA. Alternatively, DNAs encoding TNF-R proteins can be assembled by ligation of synthetic

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I 171 173  
L  
oligonucleotide subunits corresponding to all or part of the sequence of [Figures 2-3 or Figures 4-6] SEQ ID NO:1 or SEQ ID NO:3 to provide a complete coding sequence. --

Page 9, lines 20-26, delete in their entirety, and insert therefor

B4  
I 171 173  
L  
-- Using this approach, approximately 240,000 cDNAs were screened in pools of approximately 800 cDNAs until assay of one transfectant pool indicated positive foci for TNF binding. A frozen stock of bacteria from this positive pool was grown in culture and plated to provide individual colonies, which were screened until a single clone ([clone 11] clone 1) was identified which was capable of directing synthesis of a surface protein with detectable TNF binding activity. The sequence of cDNA [clone 11] clone 1 isolated by the above method is depicted in [Figures 4-6] SEQ ID NO:1. --

Page 13, lines 15-29, delete in their entirety, and insert therefor

B5  
I 171 173  
L  
-- Subunits of TNF-R may be constructed by deleting terminal or internal residues or sequences. Particularly preferred sequences include those in which the transmembrane region and intracellular domain of TNF-R are deleted or substituted with hydrophilic residues to facilitate secretion of the receptor into the cell culture medium. The resulting protein is referred to as a soluble TNF-R molecule which retains its ability to bind TNF. A particularly preferred soluble TNF-R construct is TNF-RA235 (the sequence of amino acids 1-235 of [Figure 2A] SEQ ID NO:1), which comprises the entire extracellular region of TNF-R, terminating with Asp<sup>235</sup> immediately adjacent the transmembrane region.

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171173 Additional amino acids may be deleted from the [transmembrane]  
I extracellular region while retaining TNF binding activity. For  
example, huTNF-RA183 which comprises the sequence of amino  
acids 1-183 of [Figure 2A] <sup>FIG.</sup> SEQ ID NO:1, and TNF-RA163 which  
I 171173 comprises the sequence of amino acids 1-163 of [Figure 2A] SEQ ID  
NO:1, retain the ability to bind TNF ligand as determined using  
the binding assays described below in Example 1. TNF-RA142,  
however, does not retain the ability to bind TNF ligand. This  
suggests that one or both of Cys<sup>157</sup> and Cys<sup>163</sup> is required for  
formation of an intramolecular disulfide bridge for the proper  
folding of TNF-R. Cys<sup>178</sup>, which was deleted without any apparent  
adverse effect on the ability of the soluble TNF-R to bind TNF,  
does not appear to be essential for proper folding of TNF-R.  
Thus, any deletion C-terminal to Cys<sup>163</sup> would be expected to result  
in a biologically active soluble TNF-R. The present invention  
contemplates such soluble TNF-R constructs corresponding to all or  
part of the extracellular region of TNF-R terminating with any  
amino acid after Cys<sup>163</sup>. Other C-terminal deletions, such as  
TNF-FA157, may be made as a matter of convenience by cutting TNF-R  
cDNA with appropriate restriction enzymes and, if necessary,  
reconstructing specific sequences with synthetic oligonucleotide  
linkers. The resulting soluble TNF-R constructs are then inserted  
and expressed in appropriate expression vectors and assayed for  
the ability to bind TNF, as described in Example 1. Biologically  
active soluble TNF-Rs resulting from such constructions are also  
contemplated to be within the scope of the present invention. --

Page 14, lines 1-12, delete in their entirety.

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Page 15, lines 10-21, delete in their entirety, and insert therefor

-- Both monovalent forms and polyvalent forms of TNF-R are useful in the compositions and methods of this invention. Polyvalent forms possess multiple TNF-R binding sites for TNF ligand. For example, a bivalent soluble TNF-R may consist of two tandem repeats of amino acids 1-235 of <sup>FIG.</sup> [Figure 2A] SEQ ID NO:1, separated by a linker region. Alternate polyvalent forms may also be constructed, for example, by chemically coupling TNF-R to any clinically acceptable carrier molecule, a polymer selected from the group consisting of Ficoll, polyethylene glycol or dextran using conventional coupling techniques. Alternatively, TNF-R may be chemically coupled to biotin, and the biotin-TNF-R conjugate then allowed to bind to avidin, resulting in tetravalent avidin/biotin/TNF-R molecules. TNF-R may also be covalently coupled to dinitrophenol (DNP) or trinitrophenol (TNP) and the resulting conjugate precipitated with anti-DNP or anti-TNP-IgM, to form decameric conjugates with a valency of 10 for TNF-R binding sites. --

Page 30, lines 23-29, delete in their entirety, and insert therefor

-- A cDNA encoding a soluble huTNF-RA235 (having the sequence of amino acids 1-235 of <sup>FIG.</sup> [Figure 2A] SEQ ID NO:1) was constructed by excising an 840 bp fragment from pCAV/NOT-TNF-R with the restriction enzymes Not1 and Pvu2. Not1 cuts at the multiple cloning site of pCAV/NOT-TNF-R and Pvu2 cuts within the TNF-R coding region 20 nucleotides 5' of the transmembrane region. In order to reconstruct the 3' end of the TNF-R sequences, two

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oligonucleotides (encoding amino acids corresponding to Ala<sup>229</sup>-Asp<sup>235</sup> of SEQ ID NO:1) were synthesized and annealed to create the following oligonucleotide linker:

Pvu2 BamH1 Bgl2

CTGAAGGGAGCACTGGCGACTAAGGATCCA  
GACTTCCCTCGTGACCGCTGATTCCTAGGTCTAG  
AlaGluGlySerThrGlyAspEnd

This oligonucleotide linker has terminal Pvu2 and Bgl2 restriction sites, regenerates 20 nucleotides of the TNF-R, followed by a termination codon (underlined) and a BamH1 restriction site (for convenience in isolating the entire soluble TNF-R by Not1/BamH1 digestion). This oligonucleotide was then ligated with the 840 bp Not1/Pvu2 TNF-R insert into Bgl2/Not1 cut pCAV/NOT to yield psolhuTNF-RA235/CAVNOT, which was transfected into COS-7 cells as described above. This expression vector induced expression of soluble human TNF-R which was capable of binding TNF. --

Page 31, lines 1-12, delete in their entirety.

Page 31, lines 17-37, delete in their entirety, and insert therefor \_\_\_\_\_

-- A cDNA encoding a soluble huTNF-RA185 (having the sequence of amino acids 1-185 of <sup>FIG.</sup> [Figure 2A] SEQ ID NO:1) was constructed by excising a 640 bp fragment from pCAV/NOT-TNF-R with the restriction enzymes Not1 and Bgl2. Not1 cuts at the multiple cloning site of pCAV/NO-TNF-R and Bgl2 cuts within the TNF-R coding region at nucleotide 637, which is 237 nucleotides 5' of the transmembrane region. The following oligonucleotide linkers

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(encoding amino acids corresponding to Ile<sup>162</sup>-Ala<sup>176</sup> and Val<sup>177</sup>-Arg<sup>185</sup>  
of SEQ ID NO:1) were synthesized:

Bgl2

7730X  
5'-GATCTGTAACGTGGTGGCCATCCCTGGGAATGCAAGCATGGATGC-3'  
ACATTGCACCACCGGTAGGGACCCTTACGTTCG  
IleCysAsnValValAlaIleProGlyAsnAlaSerMetAspAla

Not1

B8  
Cont  
5'-AGTCTGCACGTCCACGTCCCCACCCGGTGAGC-3'  
TACCTACGTCAGACGTGCAGGTGCAGGGGGTGGGCCACTCGCCGG  
ValCysThrSerThrSerProThrArgEnd

The above oligonucleotide linkers reconstruct the 3' end of the receptor molecule up to nucleotide 708, followed by a termination codon (underlined). These oligonucleotides were then ligated with the 640 bp Not1 TNF-R insert into Not1 cut pCAV/NOT to yield the expression vector psolTNFRA185/CAVNOT, which was transfected into COS-7 cells as described above. This expression vector induced expression of soluble human TNF-R which was capable of binding TNF. --

[ Page 32, lines 1-4, delete in their entirety. /

Page 32, lines 9-25, delete in their entirety, and insert therefor

I 171173 B9  
-- A cDNA encoding a soluble huTNF-RA163 (having the sequence of amino acids 1-163 of <sup>FIG. 2A</sup> ~~Figure 2A~~ SEQ ID NO:1) was constructed by excising a 640 bp fragment from pCAV/NOT-TNF-R with the restriction enzymes Not1 and Bgl2 as described in Example 4. The

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I  
L  
following oligonucleotide linkers (encoding amino acids corresponding to Ile<sup>162</sup>-Cys<sup>163</sup> of SEQ ID NO:1) were synthesized:

T740x  
Bgl2 Not1  
5'-GATCTGTTGAGC -3'  
ACAACCTCGCCGG  
IleCysEnd

Bgl  
Cont  
This above oligonucleotide linker reconstructs the 3' end of the receptor molecule up to nucleotide 642 (amino acid 163), followed by a termination codon (underlined). This oligonucleotide was then ligated with the 640 bp Not1 TNF-R insert into Not1 cut pCAV/NOT to yield the expression vector psolTNFRA163/CAVNOT, which was transfected into COS-7 cells as described above. This expression vector induced expression of soluble human TNF-R which was capable of binding TNF in the binding assay described in Example 1. --

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Page 32, lines 31-32, delete in their entirety, and insert therefor

I 171173  
B10  
-- A cDNA encoding a soluble huTNF-RA142 (having the sequence of amino acids 1-142 of [Figure 2A] <sup>Fig.</sup> SEQ ID NO:1) was constructed by excising a 550 bp fragment from pCAV/NOT-TNF-R with the restriction enzymes Not1 and AlwN1. AlwN1 cuts within the TNF-R coding region at nucleotide 549. The following oligonucleotide

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L  
linker (encoding amino acids corresponding to Thr<sup>132</sup>-Cys<sup>142</sup> of SEQ  
ID NO:1) was synthesized:

7750X  
Bgl2 Not1  
5'-CTGAAACATCAGACGTGGTGTGCAAGCCCTGTTAAA-3'  
CTTGACTTTGTAGTCTGCACCACACGTTCTGGGACAATTTCTAGA  
End

Bio  
Cont  
This above oligonucleotide linker reconstructs the 3' end of the receptor molecule up to nucleotide 579 (amino acid 142), followed by a termination codon (underlined). This oligonucleotide was then ligated with the 550 bp Not1/AlwN1 TNF-R insert into Not1/Bgl2 cut pCAV/NOT to yield the expression vector psolTNFRA142/CAVNOT, which was transfected into COS-7 cells as described above. This expression vector did not induced expression of soluble human TNF-R which was capable of binding TNF. It is believed that this particular construct failed to express biologically active TNF-R because one or more essential cysteine residue (e.g., Cys<sup>157</sup> or Cys<sup>163</sup>) required for intramolecular bonding (for formation of the proper tertiary structure of the TNF-R molecule) was eliminated. --

Page 33, lines 1-18, delete in their entirety. ✓

Page 35, lines 28-29, delete in their entirety. ✓

Page 36, lines 1-29, delete in their entirety, and insert therefor

B11  
-- A DNA fragment encoding TNF receptor and suitable for cloning into the yeast expression vector pIXY120 was first generated by polymerase chain reaction (PCR) amplification of the extracellular portion of the full length receptor from pCAV/NOT-TNF-R

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(ATCC 68088). The following primers (encoding amino acids corresponding in part to amino acids Leu<sup>1</sup>-Thr<sup>8</sup> and Pro<sup>225</sup>-Asp<sup>235</sup> of SEQ ID NO:1) were used in this PCR amplification:

TTG9x  
5' End Primer

5'-TTCCGGTACCTTTGGATAAAAGAGACTACAAGGAC  
Asp718->ProLeuAspLysArgAspTyrLysAsp'  
GACGATGACAAGTTGCCCCGCCAGGTGGCATTAC-3'  
AspAspAspLys<-----TNF-R----->

B11  
3' End Primer (antisense)

5'-CCCGGGATCCTTAGTCGCCAGTGCTCCCTTCAGCTGGG-3'  
BamH1>End<-----TNF-R----->

The 5' end oligonucleotide primer used in the amplification included an Asp718 restriction site at its 5' end, followed by nucleotides encoding the 3' end of the yeast a-factor leader sequence (Pro-Leu-Asp-Lys-Arg) and those encoding the 8 amino acids of the FLAG<sup>®</sup> peptide (AspTyrLysAspAspAspLys) fused to sequence encoding the 5' end of the mature receptor. The FLAG<sup>®</sup> peptide (Hopp et al., *Bio/Technology* 6:1204, 1988) is a highly antigenic sequence which reversibly binds the monoclonal antibody M1 (ATCC HB 9259). The oligonucleotide used to generate the 3' end of the PCR-derived fragment is the *antisense* strand of DNA encoding sequences which terminate the open reading frame of the receptor after nucleotide 704 of the mature coding region (following the Asp residue preceding the transmembrane domain) by introducing a TAA stop codon (underlined). The stop codon is then followed by a BamH1 restriction site. The DNA sequences encoding

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B11/Cont  
TNF-R are then amplified by PCR, substantially as described by Innis et al., eds., *PCR Protocols: A Guide to Methods and Applications* (Academic Press, 1990). --

Page 38, lines 22-29, delete in their entirety, and insert therefor

B12  
I 171 173  
-- The murine cDNA library was amplified once and a total of 900,000 plaques were screened, substantially as described in Example 2, with the human TNF-R cDNA probe. Approximately 21 positive plaques were purified, and the Bluescript plasmids containing EcoR1-linkered inserts were excised (Stratagene, San Diego). Nucleic acid sequencing of a portion of murine TNF-R clone 11 indicated that the coding sequence of the murine TNF-R was approximately 88% homologous to the corresponding nucleotide sequence of human TNF-R. A partial nucleotide sequence of murine TNF-R cDNA clone 11 is set forth in [Figures<sup>FIGS.</sup> 3A-3B] SEQ ID NO:3 and SEQ ID NO:4. --

Page 40, after line 2, insert

-- DETAILED DESCRIPTION OF THE SEQUENCE LISTING

B13  
I  
SEQ ID NO:1 and SEQ ID NO:2 show the partial cDNA sequence and derived amino acid sequence of the human TNF-R clone 1. Nucleotides are numbered from the beginning of the 5' untranslated region. Amino acids are numbered from the beginning of the signal peptide sequence. The putative signal sequence is represented by amino acid -22 to -1. The N-terminus of the mature TNF-R begins with amino acid 1. The predicted transmembrane region extends from amino acids 236-265.

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I  
B3  
COT  
SEQ ID NO:3 and SEQ ID NO:4 show the cDNA sequence and derived amino acid sequence of murine TNF-R clone 11. The putative signal peptide sequence is represented by amino acids -22 to -1. The N-terminus of the mature TNF-R protein begins with amino acid 1. The predicted transmembrane region extends from amino acids 234 to 265. --

Page 41-53, please renumber as new pages 54-66, respectively.

**IN THE CLAIMS:**

I  
Claim 18. (amended) An isolated DNA molecule encoding a protein comprising a sequence of amino acids selected from the group consisting of amino acids 1-163 of SEQ ID NO:1 and amino acids 1-233 of SEQ ID NO:3, wherein said protein is capable of binding TNF.

Claim 19. (amended) The isolated DNA molecule according to Claim 18, wherein said protein comprises amino acids 1-163 of SEQ ID NO:1.

B14  
Claim 20. (amended) The isolated DNA molecule according to Claim 18, wherein said protein comprises amino acids 1-185 of SEQ ID NO:1.

Claim 21. (amended) The isolated DNA molecule according to Claim 18, wherein said protein comprises amino acids 1-235 of SEQ ID NO:1.

Claim 22. (amended) An isolated DNA molecule encoding a protein selected from the group consisting of:

(a) a polypeptide having a sequence of amino acids comprising amino acids 1-163 of SEQ ID NO:1;

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B14 Cont  
(b) a polypeptide having a sequence of amino acids comprising amino acids 1-233 of SEQ ID NO:3; and

(c) a polypeptide identical to the polypeptides of (a) or (b) except for one or more modification(s) to the sequence of amino acids selected from the group consisting of:  
(i) inactivated N-linked glycosylation sites;  
(ii) altered KEX2 protease cleavage sites; and  
(iii) substitution or deletion of cysteine residues,

wherein said protein is capable of binding TNF.

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B15  
Claim 25. (amended) The host cell of Claim 24, wherein said host cell is selected from the group consisting of microbial cells and mammalian cells.

Claim 26. (amended) The host cell of Claim 25, wherein said mammalian cells are selected from the group consisting of L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7 cells.

Claim 27. (amended) The host cell of Claim 26, wherein said mammalian cells are CHO cells.

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Claim 29. (amended) The process of Claim 28, wherein said host cell is selected from the group consisting of microbial cells and mammalian cells.

Claim 30. (amended) The process of Claim 29, wherein said mammalian cells are selected from the group consisting of L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7 cells.

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Claim 31. (amended) The process of Claim 30, wherein said mammalian cells are CHO cells.

Claim 32. (amended) An isolated DNA molecule encoding a soluble TNF receptor protein comprising a sequence of amino acids selected from the group consisting of from about amino acid 1 to about amino acid 163 of SEQ ID NO:1 and from about amino acid 1 to about amino acid 233 of SEQ ID NO:3, wherein said soluble TNF receptor protein is capable of binding TNF protein.

Claim 33. (amended) The isolated DNA molecule according to Claim 32, wherein said soluble TNF receptor protein comprises from about amino acid 1 to about amino acid 163 of SEQ ID NO:1.

Claim 34. (amended) The isolated DNA molecule according to Claim 32, wherein said soluble TNF receptor protein comprises from about amino acid 1 to about amino acid 185 of SEQ ID NO:1.

Claim 35. (amended) The isolated DNA molecule according to Claim 32, wherein said TNF soluble receptor protein comprises from about amino acid 1 to about amino acid 235 of SEQ ID NO:1.

Claim 36. (amended) An isolated DNA molecule encoding a soluble TNF receptor protein selected from the group consisting of:

- (a) a TNF receptor polypeptide having a sequence of amino acids comprising from about amino acid 1 to about amino acid 163 of SEQ ID NO:1;
- (b) a TNF receptor polypeptide having a sequence of amino acids comprising from about amino acid 1 to about amino acid 233 of SEQ ID NO:3; and

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(c) a TNF receptor polypeptide identical to the TNF receptor polypeptides of (a) or (b) except for one or more modification(s) to the sequence of amino acids selected from the group consisting of: (i) inactivated N-linked glycosylation sites; (ii) altered KEX2 protease cleavage sites; and (iii) substitution or deletion of cysteine residues,

wherein said soluble TNF receptor protein is capable of binding TNF.

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B17  
Claim 39. (amended) The host cell of Claim 38, wherein said host cell is selected from the group consisting of microbial cells and mammalian cells.

Claim 40. (amended) The host cell of Claim 39, wherein said mammalian cells are selected from the group consisting of L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7 cells.

Claim 41. (amended) The host cell of Claim 40, wherein said mammalian cells are CHO cells.

I  
B18  
Claim 43. (amended) The process of Claim 42, wherein said host cell is selected from the group consisting of microbial cells and mammalian cells.

Claim 44. (amended) The process of Claim 43, wherein said mammalian cells are selected from the group consisting of L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7 cells.

Claim 45. (amended) The process of Claim 44, wherein said mammalian cells are CHO cells.

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I Claim 46. (amended) An isolated DNA molecule encoding a soluble TNF receptor protein comprising a sequence of amino acids selected from the group consisting of from amino acid 1 to amino acid 163 of SEQ ID NO:1 and from amino acid 1 to amino acid 233 of SEQ ID NO:3, wherein said soluble TNF receptor protein is capable of binding TNF protein.

Claim 47. (amended) The isolated DNA molecule according to Claim 46, wherein said soluble TNF receptor protein comprises from amino acid 1 to amino acid 163 of SEQ ID NO:1.

Claim 48. (amended) The isolated DNA molecule according to Claim 46, wherein said soluble TNF receptor protein comprises from amino acid 1 to amino acid 185 of SEQ ID NO:1.

Claim 49. (amended) The isolated DNA molecule according to Claim 46, wherein said soluble TNF receptor protein comprises from amino acid 1 to amino acid 235 of SEQ ID NO:1.

Claim 50. (amended) An isolated DNA molecule encoding a soluble TNF receptor protein selected from the group consisting of:

- (a) a TNF receptor polypeptide having a sequence of amino acids comprising from amino acid 1 to amino acid 163 of SEQ ID NO:1;
- (b) a TNF receptor polypeptide having a sequence of amino acids comprising from amino acid 1 to amino acid 233 of SEQ ID NO:3; and
- (c) a TNF receptor polypeptide identical to the TNF receptor polypeptides of (a) or (b) except for one or more modification(s) to the sequence of amino acids selected from the group consisting



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of: (i) inactivated N-linked glycosylation sites; (ii) altered KEX2 protease cleavage sites; and (iii) substitution or deletion of cysteine residues,

wherein said soluble TNF receptor protein is capable of binding TNF.

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B19  
Claim 53. (amended) The host cell of Claim 52, wherein said host cell is selected from the group consisting of microbial cells and mammalian cells.

Claim 54. (amended) The host cell of Claim 53, wherein said mammalian cells are selected from the group consisting of L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7 cells.

Claim 55. (amended) The host cell of Claim 54, wherein said mammalian cells are CHO cells.

I  
B20  
Claim 57. (amended) The process of Claim 56, wherein said host cell is selected from the group consisting of microbial cells and mammalian cells.

Claim 58. (amended) The process of Claim 57, wherein said mammalian cells are selected from the group consisting of L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7 cells.

Claim 59. (amended) The process of Claim 58, wherein said mammalian cells are CHO cells.

Claim 60. (amended) An isolated DNA molecule encoding a protein comprising a sequence of amino acids selected from the group consisting of amino acids 1-163 of SEQ ID NO:1 and amino acids 1-233 of SEQ ID NO:3, wherein said protein lacks amino

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acids 236-265 of SEQ ID NO:1 and amino acids 234-265 of SEQ ID NO:3, respectively, and wherein said protein is capable of binding TNF.

Claim 61. (amended) The isolated DNA molecule according to Claim 60, wherein said protein comprises amino acids 1-163 of SEQ ID NO:1.

Claim 62. (amended) The isolated DNA molecule according to Claim 60, wherein said protein comprises amino acids 1-185 of SEQ ID NO:1.

Claim 63. (amended) The isolated DNA molecule according to Claim 60, wherein said protein comprises amino acids 1-235 of SEQ ID NO:1.

Claim 64. (amended) An isolated DNA molecule encoding a protein selected from the group consisting of:

- (a) a TNF receptor polypeptide having a sequence of amino acids comprising amino acids 1-163 of SEQ ID NO:1, wherein said polypeptide lacks amino acids 236-265 of SEQ ID NO:1;
- (b) a TNF receptor polypeptide having a sequence of amino acids comprising amino acids 1-233 of SEQ ID NO:3, wherein said polypeptide lacks amino acids 234-265 of SEQ ID NO:3; and
- (c) a TNF receptor polypeptide identical to the TNF receptor polypeptides of (a) or (b) except for one or more modification(s) to the sequence of amino acids selected from the group consisting of: (i) inactivated N-linked glycosylation sites; (ii) altered KEX2 protease cleavage

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*B20*  
*cont*  
sites; and (iii) substitution or deletion of  
cysteine residues,

wherein said protein is capable of binding TNF.

*I*  
Claim 67. (amended) The host cell of Claim 66, wherein said  
host cell is selected from the group consisting of microbial  
cells and mammalian cells.

*B21*  
Claim 68. (amended) The host cell of Claim 67, wherein said  
mammalian cells are selected from the group consisting of  
L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7  
cells.

Claim 69. (amended) The host cell of Claim 68, wherein said  
mammalian cells are CHO cells.

*I*  
Claim 71. (amended) The process of Claim 70, wherein said  
host cell is selected from the group consisting of microbial  
cells and mammalian cells.

*B22*  
Claim 72. (amended) The process of Claim 71, wherein said  
mammalian cells are selected from the group consisting of  
L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7  
cells.

Claim 73. (amended) The process of Claim 72, wherein said  
mammalian cells are CHO cells.

Claim 74. (amended) An isolated DNA molecule encoding a  
protein comprising a sequence of amino acids selected from the  
group consisting of amino acids 1-163 of SEQ ID NO:1 and amino  
acids 1-233 of SEQ ID NO:3, wherein said protein lacks a  
functional transmembrane region, and wherein said protein is  
capable of binding TNF.

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Claim 75. (amended) The isolated DNA molecule according to Claim 74, wherein said protein comprises amino acids 1-163 of SEQ ID NO:1.

Claim 76. (amended) The isolated DNA molecule according to Claim 74, wherein said protein comprises amino acids 1-185 of SEQ ID NO:1.

Claim 77. (amended) The isolated DNA molecule according to Claim 74, wherein said protein comprises amino acids 1-235 of SEQ ID NO:1.

Claim 78. (amended) An isolated DNA molecule encoding a protein selected from the group consisting of:

(a) a TNF receptor polypeptide having a sequence of amino acids comprising amino acids 1-163 of SEQ ID NO:1;

(b) a TNF receptor polypeptide having a sequence of amino acids comprising amino acids 1-233 of SEQ ID NO:3; and

(c) a TNF receptor polypeptide identical to the TNF receptor polypeptides of (a) or (b) except for one or more modification(s) to the sequence of amino acids selected from the group consisting of: (i) inactivated N-linked glycosylation sites; (ii) altered KEX2 protease cleavage sites; and (iii) substitution or deletion of cysteine residues.

wherein said protein lacks a functional transmembrane region; and wherein said protein is capable of binding TNF.

SUPPLEMENTAL PRELIMINARY AMENDMENT  
U.S. Appln. No. 09/144,502

I  
§23  
Claim 81. (amended) The host cell of Claim 80, wherein said host cell is selected from the group consisting of microbial cells and mammalian cells.

Claim 82. (amended) The host cell of Claim 81, wherein said mammalian cells are selected from the group consisting of L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7 cells.

Claim 83. (amended) The host cell of Claim 82, wherein said mammalian cells are CHO cells.

I  
§24  
Claim 85. (amended) The process of Claim 84, wherein said host cell is selected from the group consisting of microbial cells and mammalian cells.

Claim 86. (amended) The process of Claim 85, wherein said mammalian cells are selected from the group consisting of L cells, C127 cells, 3T3 cells, CHO cells, BHK cells and COS-7 cells.

Claim 87. (amended) The process of Claim 86, wherein said mammalian cells are CHO cells.

REMARKS

The specification is being amended in a manner consistent with 37 C.F.R. § 1.121(b)(1)(iii) as requested by the Examiner in a teleconference on April 16, 1999.

The above-noted amendments to the specification are identical to those previously submitted in the Preliminary Amendment filed October 9, 1998, which the Examiner has advised he has not entered because they were not made in accordance with